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Practical application of sea urchin shell flour supplementation as a stimulant moulting in vannamei shrimp

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ABSTRACT

The practical application of waste recycling as feed supplement is potentially required by small-scale aquaculturist. Furthermore, some of the flour derived from waste sea urchin shells and in adopted in feed, including *Deadema setosum* were evaluated to estimate the effect on white shrimp *Litopenaeus vannamei* moulting. This research required the experimentation of four doses with triplicate supplementation (0, 2, 4, and 6 g 100 g^{-1} of feed) on shrimps four times daily (12% of body weight) for 35 days test period. The juvenile specimens, characterized by an initial weight of 1.61±0.11 g, were stocked at a density of 12 individuals in a 12 L aquarium. In addition, four compartments were created in each aquarium to facilitate progress observations. The proximate analysis results showed a 53.76±0.27% calcium content in the shell flour, which significantly increased ($P<0.05$) after higher dose supplementation, in the sequential order 18.65±0.13%, 20.04±0.08%, 23.18±0.10%, and 25.04±0.11%. Moreover, the frequency and moulting interval with 4 g doses (16.59%±0.36% day^{-1} and 5.91±0.18 $days^{-1}$) were significant ($P<0.05$) and considered the best, compared to 0 g (10.48% ± 0.24% day^{-1} and 9.97±0.37 $days^{-1}$), 2 g (13.49%±0.96% day^{-1} and 8.10±0.29 $days^{-1}$), and 6 g (13.81%±0.24% day^{-1} and 7.90±0.06 $days^{-1}$). In addition, the respective trend pattern for both parameters increased and decreased at 4 g and 6 g, correspondingly. The highest moulting intensity was also obtained with the 4 g doses, at a range of 4 to 6 times, while the lowest (0 g) varied from 3 to 4 times. These sea urchin shell flour was determined to have numerous practical applications as a feed supplement with proven ability to stimulate moulting in vannamei shrimp.

Introduction

Moulting or shell changing is a necessary condition for crustacean growth. This process occurs periodically throughout the life of the organism (Chang and Mykles, 2011; Hou *et al.*, 2011). The crustaceans' body surface is covered by an exoskeleton or rigid shell, which is shed periodically (Corteel *et al.*, 2012), as a form of adjustment to the increased meat size (Huner, 2003; Hosamani, *et al.*, 2017). Subsequently, the old shell is removed and replaced with one characterized by an appropriate dimension to the increased body size.

Moulting is an important event in the physiology of crustaceans (Chang, 1995; Corteel *et al.*, 2012; Lemos and Weissman, 2021), and is characterized by 4 phases, including pre-molt, molt, post-molt, and inter-molt (Merrick, 1993; Chang, 1995; Corteel *et al.*, 2012). Furthermore, calcium and magnesium are required to support the activities in each phase, due to the importance in shell hardening (Tavabe *et al.*, 2013; Nurussalam *et al.*, 2017; Lemos and Weissman, 2021). Moreover, both tend to also play a role in physiological homeostasis, osmoregulation nerve transmission, muscle contraction, protein

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synthesis for growth, and as cofactors in metabolic processes (Li and Cheng, 2012; Jannathulla *et al*., 2020). The success of moulting depends on the sequential levels of calcium obtained through all phases (Zaidy and Hadie, 2009).

The process is known to ensue naturally or under conditions stimulated by the addition of certain minerals. This artificial procedure is performed to accelerate shell hardening, moulting frequency, and consequently shrimp growth. In addition, stimulation treatments have been widely reported, including the administration of calcium oxide (CaO) from crab (*Scylla serrata*) shells (Zufadhillah *et al*., 2014; Fajri *et al*., 2019), devil lava snail *(Faunus ater)* shells (Handayani *et al*., 2019), and lime hydrocide ($Ca(OH)_2$) (Zaidy and Hadie, 2009) to feed and water. These potentially increase the moulting frequency of giant freshwater prawn *(Macrobrachium rosenbergii)*. Moreover, similar outcome is observed with the addition of calcium oxide (CaO) from oyster *(Crassostrea gigas)* shells to the feed of freshwater lobster *(Cherax quadricarinatus)* (Handayani and Syahputra, 2018). This treatment in combination magnesium sulfate ($MgSO_4$) potentially increases the amount of mud crab (*Scylla serrata)* (Nurussalam *et al*., 2017).

There have been numerous studies on calcium application for moulting in vannamei shrimp. However, the addition of dicalcium phosphate ($CaHPO_4$) (Kaligis, 2015), calcium chloride ($CaCl_2$) (Sirin and Mazlum, 2017), and dolomite (CaMg $(CO3)_2$) (Aisyah *et al*., 2017) to feed is known to help accelerate moulting. Moreover, the administration of calcium oxide (CaO) (Erlando *et al*, 2016), calcium carbonate ($CaCO_3$) (Heriadi, *et al*., 2013), and lime hydrocide ($Ca(OH)_2$) (Yulihartini, *et al*., 2017) in water positively influences moulting. In addition, a combination of dolomite $(CO3)_2$ and quicklime (CaO) (Yunus, *et al*., 2020) alongside calcium ions Ca^{2+} (Hou *et al*., 2011) in water potentially increases the amount and frequency moulting in vannamei shrimp.

Previous studies have confirmed a successful moulting stimulation in crustaceans, following the addition of calcium from various sources at specified dosed. Kaligis (2015) reported the highest frequency in vannamei shrimp at a dose of 4 g of dicalcium phosphate (per 100 g of feed) compared to 0 g and 2 g. Moreover, the most significant value determined in a study by Sirin and Mazlum (2017) on lobsters was obtained at a dose of 6 g calcium chloride (per 100 g of feed) compared to 0 g, 3 g, and 12 g. Therefore, it is necessary to explore the potentials of other alternative calcium sources. The specific requirements for selection include being a fishery byproduct with significant reserves, and a substantial ease of application on a field scale.

Sea urchins are known to play an important role as foodstuffs in various countries, and are characterized by delicious gonads, high nutrition as well as cost (Arafa *et al*., 2012; Baião *et al*., 2019; Nane and Paramata, 2020). These economic values, including the potential to produce shell waste have led to high gonad demand. In addition, the dry weight of sea urchins *D. setosum* 610 g are dominated by 46.72% shells, 17.37% spines, 16.88% comprise other parts, and only 8.03% of gonads (Akerina *et al*., 2015). There is high proportion of recyclable material as a potential waste for added value.

Furthermore, the shell of the sea urchin is well-known to have high calcium content. The main constituents include minerals, encompassing calcium carbonate, magnesium, and others with (Politi *et al*., 2004; Akerina *et al*., 2015; Cahyono *et al*., 2019). These structures comprise about 90.77-93.10% of Ca, Mg, Na, K, Mn, Zn, P and Fe with calcium and magnesium being the most significant, at 56.23% and 39.97%, respectively. In addition, 5.24% carbohydrates and 4.06-4.99% proteins with amino acids 94.88-96.79% have also been recorded (Amarowicz *et al*., 2012; Addina, 2016).

These intrinsic potentials have been implicated in some negative impacts, particularly from the environmental, aesthetic and hygienic perspective. The waste products have been evaluated to have recycling applications in flour production for addition as supplements. This study adopted the practical approach of utilizing sea urchin shell as a stimulant in moulting of vannamei shrimp, which is currently considered the most important amongst similar species in national and global aquaculture. The purpose of this research, therefore, was to evaluate the effect of sea urchin shell flour supplementation in feed on the moulting performance of vannamei shrimp. Therefore, the results are expected to be applied by farmers, and are also required to provide basic knowledge for further research on the potentials of sea urchin shells in aquaculture activities.

Materials and Methods

Time and site

The research was conducted for 28 days in the Field Laboratory of the Brackish water Farm, Balik Diwa Institute of Maritime Technology and Business, Makassar, Indonesia. Subsequently, calcium levels in the shell flour were analyzed at the Nutrition Laboratory, State Agricultural Polytechnic, Pangkep.

Experimental design

A total of four experimental dosages were prepared to supplement the flour sea urchin shell, including 0 (control), 2, 4, and 6 g 100 $g^{-1,}$ and each was prepared in three replicates. The respective doses were applied at an interval of 2 g dicalcium phosphate, considering 4 g as the optimum value required to stimulate moulting in *L. vannamei* (Kaligis, 2015). Therefore, similar amounts were administered during this study in attempts to evaluate the upper and lower limits of the doses.

Furthermore, *D. setosum* shell as a source of calcium was collected from the coast of Saugi Island, Pangkep Regency. The 144 *L vannamei* juveniles were obtained from a hatchery in Talaka Village Pangkep Regency, and acclimated to the experimental conditions for 3 days. This period was characterized by feeding to satiation four times a day (at 7 a.m., 12 a.m., 5 p.m., 10 p.m.), with a commercial feed, characterized by 37% crude protein, 6% fat, 2.5% fiber, and 12% ash. Consequently, the specimen were exposed to and maintained at a temperature of 29-31°C, 10-15 ppt salinity, >4 mg L^{-1}dissolved oxygen, and pH of 7.5-8.0.

The experimental feeds were prepared practically by first cutting the sea urchin spines and removing the gonads. Therefore, the shells were washed clean and dried in the sun. Subsequently, the samples were chopped with a blender and filtered through a 60 mesh. Therefore, shell flour was then supplemented into commercial feed by a coating process, involving the homogeneous mixing with 10 mL of water. Furthermore, the product was supplemented to 100 g with feed, where 2% egg whites served as a binder. Finally, the output was wind-dried and ready to be applied, as shown in Figure 1.

The experiments were conducted in an aquarium with specified dimensions (40 x 20 x 20 cm), and set up as shown in Figure 2. In addition, each unit was filled with 12 L of brackishwater characterized by similar quality parameters at the acclimation tanks. The 144 juveniles with a mean body weight of 1.61 ± 0.11 g were randomly collected from the acclimation tank and stocked at 12 individuals per aquarium. In addition, four compartments were created in each using nets partitions to facilitate monitoring, equipped with 1 aeration point and 2 PVC pipes (diameter of 1 inch and length of 10 cm) as shelters, and a total of three (3) individuals were housed in each.

The juveniles had an initial weight of 1.61 ± 0.11 g during the experimental period (35 days), and were feed at a rate of 12% body weight, through similar time interval as observed during the acclimation period. This percentage is a medium feeding rate for vannamei shrimp (Khanjani *et al.*, 2016), and the was administered proportionally to each compartment. Subsequently, the uneaten feed and feces were siphoned off after 4 hours, in relation to the stomach empty rate, ranging from 2-4 hours (Dall *et al.*, 1990). The amount of water lost was then replaced. Furthermore, about 20% of the entire water in each aquarium was changed every 7 days, and the respective test units were covered with a black net, to prevent the specimens from jumping out.

Figure 1. Shells and flour shells of *D. setosum.*

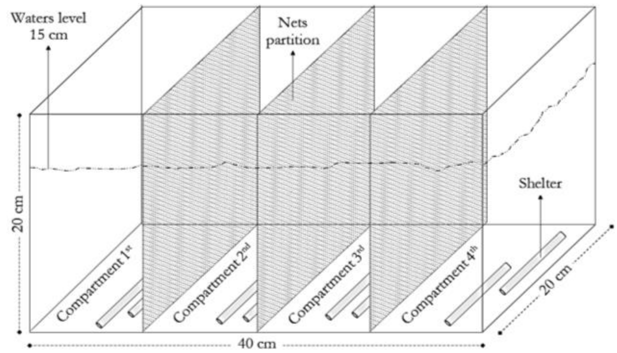

Figure 2. Design of aquarium.

Data collection procedure

The research was conducted by observing the moulting performance of shrimp. This involved determining the frequency, interval, and intensity during the study period. Particularly, Moulting frequency (MF) were calculated as follows: MF (% day^{-1}) = 100 x N_m / (N_s x T) (Hou *et al.*, 2011), where, N_m is the number of molts; N_s is the number of juveniles, T is the duration of the experiment. The interval and intensity data were collected by calculating the time interval between the first moulting and the next moulting. This also required counting the number of times moulting occurred on each sample. Furthermore, water quality parameters were measured daily to ensure the values were within the tolerance limits for *L. vannamei.*

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Analysis data

Analysis of variance (one-way Anova) and Tukey's HSD were conducted at a significant rate of 95% ($p<0.05$) to determine the difference in moulting performance per treatment. Subsequently, water quality data were analyzed descriptively-comparative, based on the standards for *L. vannamei* culture.

Results

The calcium level in shell flour was determined using the atomic absorption spectrometry method as 53.76 ± 0.27%. Table 1 shows the respective concentration in the experimental feed of each treatment. Furthermore, the highest value (25.04 ± 0.11%) was obtained at a supplementation doses of 6 g, with the least in 0 g (control) (18.65 ± 0.131%). The calcium level in 0 g supplementation feed denotes the commercial variant. Also, the trend pattern tends to increase at higher supplementation doses. The one-way Anova results showed significant effects and difference at each concentration ($P<0.05$).

Table 2 shows the variations in moulting frequency, interval, and intensity with each treatment. Particularly, the frequency and moulting interval at the supplementation doses of 4 g treatment group was significantly ($P<0.05$) higher than any the other. However, no significant difference ($P>0.05$) was recorded between 2 g and 6 g. The trend pattern for both parameters increased up to treatments with 4 g, and a subsequent decline was observed at 6 g. Furthermore, the highest intensity (4-6 times) was attained at 4 g, while the lowest outcome (3-4) was observed with the control (0 g). Table 3 shows water quality data measured during the experiment to be within a range tolerable by *L. vannamei*.

Table 1. Calcium level of supplementation feed.

Treatments	Calcium levels (%) (mean ± SD)
0 g	18.65 ± 0.13^{a}
2 g	20.04 ± 0.08^{b}
4 g	23.18 ± 0.10^{c}
6 g	25.04 ± 0.11^{d}

The different superscript letters in the same column show significant differences ($P<0.05$) by Tuke'y HSD.

Table 2. Performance moulting of each treatments.

Parameters	Treatments			
	0 g	2 g	4 g	6 g
Frequency of moulting (% day^{-1})	10.48 $\pm 0.24^{a}$	13.49 $\pm 0.96^{b}$	16.59 $\pm 0.36^{c}$	13.81 $\pm 0{,}24^{b}$
Interval of moulting (days)	9.97 $\pm 0.37^{a}$	8.10 $\pm 0.29^{b}$	5.91 $\pm 0.18^{c}$	7.90 $\pm 0.06^{b}$
Intensity of moulting (times)	3-4	3-5	4-6	4-5

The different superscript letters in the same row show significant differences ($P<0.05$) by Tukey HSD

Table 3. Water quality measurement data.

Parameters	Ranges
Temperature (°C)	29.1-31.3
Salinity (ppt)	13.3-14.2
Dissolved oxygen (mg L^{-1})	4.76-4.96
pH	7.2-7.3

Discussion

Calcium levels

The calcium level in shell flour *D. setosum* was 53.76 ± 0.27%. This result obtained in previous researches by (Addina, 2016) and (Cahyono *et al.*, 2019), where 56.23 ± 0.13% and 25.53 ± 2.50%, respectively, following the evaluation of similar sea urchin species. In addition, the marked discrepancies in value is assumed to occur due to differences in the flour making process, as well as in species and habitats variations.

The high calcium levels obtained in this study further strengthen the potential application of sea urchin shells as a natural source of calcium (Politi *et al.*, 2004; Akerina *et al.*, 2015; Cahyono *et al.*, 2019). This is attributed to the presence of calcium carbonate (calcite $CaCO_3$), identified as one of the main substances in shells, and magnesium carbonate (magnesite $MgCO_3$) (Shapkin *et al.*, 2017). Furthermore, both constituents have been identified at the prismatic layer, and are known to occur in the form of calcite and aragonite crystals (Amarowicz *et al.*, 2012). Table 1 shows the total calcium present in the diet, which increased at higher supplementation doses. Moreover, the homogenization between shell flour and feed, using egg whites as binder ensures a proper calcium blend.

There is high demand for calcium during shell formation and hardening in the moulting phase. This process is expected to not only rely on supplies present in waters, which varies and fluctuates in relation to numerous environmental influences (Li and Cheng, 2012), including rain and tides. Therefore, it is essential to investigate alternative additional sources, required to increase the amount available in the aquatic environment. In addition, small-scale shrimp farmers with frequent limitations require practical and easily applied technological cultivation methods. Hence, this study outcome is expected to serve as an information source for applicative options on a field scale to stimulate shrimp moulting. Simultaneously, the shell wastes are used to generate added value.

Moulting performance

Several previous studies have utilized calcium for moulting purposes. However, this is the first study to evaluate the use of sea urchin shell flour, as a moulting stimulant. Calcium addition is conducted through feed and water, therefore, this article's initial discussion begins with an explanation of the calcium addition choice, feed supplementation.

Furthermore, several studies show additive administered through feed is effectively absorbed by the organism. Hepatopancreas are the moulting cycle's most important organ (Chang, 1995; Li and Cheng, 2012; Jannathulla *et al*., 2020), and this organ receives food allowing ingested substances' assimilation into the body (Al-Mohanna and Nott, 1989). Suguna (2020) reported macro mineral inclusion through feed possibly increases the vannamei shrimp's growth. Shrimp absorb calcium through gills, however, supplementation in diet is important, especially in cases where the supplemented calcium is sourced from animals (Moss *et al*., 2019), and this is related to *L. vannamei*'s carnivorous feeding habits. A study by Handayani *et al*. (2019) further confirms calcium addition from oyster shells in the feed results in a higher moulting frequency in crayfish, compared to addition through water media. Similarly, Hakim (2012) stated the feed addition method (oral method) is more effective for moulting, compared to water medium (deeping method). Calcium is directly digestible with feed, and is therefore readily always available for moulting.

In addition, calcium absorbed through feed stimulates shrimp to moult by the physiological mechanisms below. Moulting is controlled by MIH (Molt Inhibiting Hormone) in organ X, with the ability to regulate and inhibit moulting hormone (ecdysteroid) secretion from organ Y (Spaziani *et al*., 1999; Huberman, 2000; Nakatsuji *et al*., 2006). Meanwhile, calcium stimulates ecdysteroid production by activating phospho-diesterase (PDE) (Mattson and Spaziani, 1986; Spaziani *et al*., 1999), and increased PDE activity contributes to reduced MIH response (Spaziani *et al*., 1999; Nakatsuji *et al*., 2006; Chang and Mykles, 2011), thus, increasing ecdysteroid release (Chen *et al*., 2020). This is therefore a logical mechanism to explain calcium's mechanism in stimulating *L. vannamei* moulting in this study.

Calcium addition through feed supplementation is also possibly used to minimize the impact of low salinity due to rainfall or pond locations. Furthermore, rainfall tends to reduce pH, and in some cases, shrimp undergo mass moulting. Simultaneously, rainfall decreases salinity, thus reducing calcium availability in the waters. Similarly, the pond's distance from the coast leads to a higher reduction in salinity. In low salinity ponds, shrimp have difficulty maintaining mineral balance, and this tends to result in mortality during moulting (Lemos and Weissman, 2021). Within these conditions, calcium addition through feed is a practical application to supply the shrimp's calcium needs. In principle, the feed addition method is also the addition of calcium to water. Supplementations in feed used egg whites as a binder, but leaching seemed to occur in this study, as indicated by the water color becoming more turbid in the calcium supplemented treatments. However, leaching calcium is likely absorbed by *L. vannamei* through the osmotic mechanism (Li and Cheng, 2012; Lemos and Weissman, 2021). Table 3 shows leaching calcium absorption indication is the water's narrow pH range. Thus, absorption from both feed and water is possible in cases where leaching occurs, and this is one benefit of the supplementation method.

The discussion below is related to moulting performance obtained in this study. Generally, *L. vannamei*'s moulting performance is significantly influenced by the calcium supplementation's dose (Table 2). Also, there is a tendency pattern of moulting performance at a 4 g dose, as a culmination point, and similar patterns were reported in several previous vannamei moulting studies. Hou *et al*. (2011) reported an increased moulting frequency at concentrations of Ca^{2+} 385, 591 to 803 mg L^{-1} (5.1%, 7.0%, 7.5% day^{-1}), but decreased at 1155 and 2380 mg L^{-1} (7.3 and 6.9% day^{-1}). The same pattern was found in the study by Erlando *et al*. (2016), where the total moulting shrimp initially increased at a doses of CaO 25, 50 to 75 mg 20 L^{-1} (8.67, 9.33 and 14.3 individuals) and decreased at 100 mg 20 L^{-1} (9.33 individuals). Similarly, Heriadi *et al*. (2013) obtained a rise in moulting shrimp numbers, starting at doses of CaCO3 20, 35, 50 to 65 mg L^{-1} (45.0, 50.7, 66.7, 136.0 individuals), and subsequently, decreasing at 80s mg L^{-1} (67.3 individuals). Studies by Yulihartini *et al*. (2017) using $Ca(OH)_2$, and Yunus *et al*. (2020) using a $CaMg(CO_3)_2$ and CaO combination, also obtained a similar pattern with this study, indicating there is a maximum calcium level required by crustaceans for moulting.

In this study, the maximum level calcium of sea urchin shell flour for *L. vannamei* moulting seem to be 4 g 100 g^{-1} of feed. Similarly, Kaligis (2015) reported a maximum dicalcium phosphate level for *L. vannamei* moulting was 4 g per 100 g^{-1} of feed. Conversely, the studies by Sirin and Mazlum (2017) reported the maximum calcium chloride doses for lobster moulting was 6 g 100 g^{-1} of feed. These differences are most likely due to the different calcium sources, species, and experimental conditions. However, further research on calcium uptake in shells and calcium reserves in the hepatopancreas are required for certainty. This indication is consistent with an optimal point concept, where the highest limit is the maximum limit producing the best effect. According to Tavabe *et al.* (2013), extremely high or low calcium levels are bound to have a negative effect on crustaceans. Suguna (2020) also warned excessive intake of one mineral is bound to produce poor results for *L. vannamei* shrimp.

The optimal calcium doses allow a balance between availability and vannamei shrimp requirements, in a bid to accelerate the moulting physiological process, especially in the pre-molt and post-molt phases (Li and Cheng, 2012). In the pre-molt phase, a calcification process or an osmotic calcium inorganic salts absorption from old skin, feed, and waters, as a reserve in the hepatopancreas organ, while the post-molt phase involves a calcium transfer and new exoskeleton hardening (Merrick, 1993; Chang and Mykles, 2011; Corteel *et al.*, 2012). In this phase, the calcium source for shell hardening is derived more from hepatopancreas, and only a small part of the waters (Affandi and Tang, 2017). The optimal calcium absorbed by shrimp is bound to accelerate calcification, thus, old shell separation and new shell hardening becomes faster. Also, the postmolt duration is reduced, thus, accelerating the next moulting period (intermolt). This condition is enabled at a dose of 4 g, as well as the best moulting frequency, interval, and intensity, compared to other doses in this study. Irawan (1988) stated shrimp shell hardening is bound to occur 4 hours faster for normal calcium levels, and 6 to 9 hours slower for lower levels.

Moulting performance at doses of 2 and 6 g, is the calcium supply's impact in both doses. At a 2 g dose, the calcium supply is considered insufficient, thus, moulting performance is inoptimal because the shell formed is thinner, old shell separation and new shell hardening durations are longer, and energy is widely used to maintain the balance between calcium in the body and water (Li and Cheng, 2012; Tavabe *et al.*, 2013; Jannathulla *et al.*, 2020; Lemos and Weissman, 2021). Conversely, calcium supply at a 6 g dose is considered excessive, thus, there is a deposition of minerals in the shell with the capacity to delay moulting (Jaganmohan and Kumari, 2018). A study by Adegboye (1981) disclosed low calcium levels (hypoionic) are bound to complicate shell formation, while high levels (hyperionic) complicate the calcium ions' homeostatic process. Calcium hypoionic or hyperionic conditions are bound to complicate the body's calcium ions balance with the environment, thus, making the energy for this process' continuity greater, with an impact on the moulting performance.

In this study, the sea urchin shells' relatively complete nutritional content is believed to have contributed to moulting performance. Normal physiological processes of shrimp depend on the availability of anions (bicarbonate, carbonate, chloride, and sulfate) as well as certain cations (calcium, magnesium, potassium, and sodium) (Roy *et al.*, 2007). In this study, the sea urchin shell's magnesium content of 39.97% (Addina, 2016), is also considered to contribute to the *L. vannamei*'s moulting performance, in addition to the calcium content. These two minerals are essential for skin turnover and new shell formation in vannamei shrimp (Suguna, 2020). Also, cannibalism often occurring during moulting is resolved by the shelter at each compartment. During this time, the shrimp's condition is very weak, however, the shelter provides safety.

Water quality

In principle, cultivation activities must provide a living environment with water quality in accordance with the organism's needs. In this study, the water quality parameters measured were temperature, salinity, dissolved oxygen, as well as pH, and these were generally in the optimal range (Table 3). The optimum temperature, salinity, dissolved oxygen and pH levels for *L. vannamei* are 26-32°C, 15-25 ppt, 4-6 mg L^{-1}, and 7.5-8.5, respectively (Haliman and Adijaya, 2005). Thus, water quality fluctuation, usually the main problem in moulting prediction and control (Lemos and Weissman, 2021), do not seem to pose a challenge in this study, as the values' fluctuations were all in narrow ranges.

This study is limited because the water calcium levels not measured. However, Yarish and Edwards, (1980) reported levels of 135-143 mg L^{-1}, at a 13-14 ppt salinity. In this study, the rearing media's salinity's fluctuations were narrow during the experiment, thus, enabling the water calcium level to be relatively the same. Therefore, the effect of water calcium level on moulting performance in this study is probably negligible.

Conclusion

Calcium supplementation in feed, using sea urchin shell, significantly affects the moulting performance of *L. vannamei*. Also, introducing 4 g 100 g^{-1} feed is considered optimal to stimulate the frequency, interval, and intensity. The results show practical applications, and further research is required to ensure absorption data on the shells and hepatopancreas of *L. vannamei* is collected. Also, the calcium level in the aquarium water has to be further investigated to estimate the potential effects of leaching from feed.

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